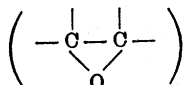


Reactions of Fatty Materials With Oxygen. IV.¹ Quantitative Determination of Functional Groups²

H. B. KNIGHT and DANIEL SWERN, Eastern Regional Research Laboratory,³
Philadelphia 18, Pennsylvania

ALTHOUGH it is generally agreed that the conventional analytical procedures for the quantitative determination of functional groups are unreliable when applied to unfractionated oxidation reaction mixtures (4, 6, 9, 24, 25), the basis for such a broad generalization is not readily ascertained from the literature. Except in a few isolated cases the magnitude of the errors has not been reported, nor has any systematic attempt been made to demonstrate which analytical methods are reliable and which are not. In studying the mechanisms of the reaction of fatty materials with oxygen, it is essential to determine accurately the distribution of absorbed oxygen among the various types of oxygen-containing groups known to be present, and it is also essential that the sum of the percentages of oxygen determined analytically on the mixture equals the known oxygen content of the material determined by combustion analysis. As will be shown later, in certain types of mixtures the sum of the percentages of oxygen determined by conventional techniques considerably exceeds the known oxygen content.

In a previous publication (20) we described a procedure for the determination of oxirane oxygen



and indicated which functional groups in oxidation mixtures would interfere. The present investigation had three objectives: a) To prepare pure compounds containing one or, at most, two functional groups encountered in oxidation reaction mixtures and to

analyze each of these compounds by the conventional techniques employed in studying oxidation reactions (acid number, saponification number, iodine number, oxirane oxygen, hydroxyl oxygen, carbonyl oxygen, and peroxide oxygen). In this way the magnitude of the interference (if any) of each functional group on each of the analytical methods could be readily determined. b) To prepare synthetic mixtures of known composition from these pure compounds and re-analyze the mixtures to determine the combined effect of numerous functional groups on the analytical techniques. c) When possible, to modify and improve those analytical procedures found to be in error, thus permitting a more accurate accounting of the total oxygen content. It is a sad commentary on research in this field that in more than 100 years of intensive study, resulting in numerous publications, a systematic investigation of this type has not been previously published.

Experimental

(All distillations were conducted in an atmosphere of nitrogen.)

Preparation of Pure Materials. Methyl oleate, b.p. 181-2°/3.6 mm. and n_D^{20} 1.4485, was prepared by fractional crystallization of olive oil fatty acids, followed by esterification with methanol and fractional distillation (1, 21, 27). 9,10-Epoxystearic acid, m.p. 59.6-59.8°, was prepared from pure oleic acid by epoxidation with peracetic acid (5). 9,10-Dihydroxystearic acid, m.p. 95°, was prepared from oleic acid by hydroxylation with hydrogen peroxide in acetic acid solution (19). Methyl ricinoleate, b.p. 187°/2 mm. and n_D^{20} 1.4592, was prepared by fractional distillation of the methyl esters obtained by methanolysis of castor oil (11). Oleyl alcohol, m.p. 7-8°, b.p. 188-90°/4.6 mm. and n_D^{40} 1.4540, was prepared from

¹ The previous papers in this series are references 20, 22, and 23.

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³ One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

the commercial grade by low-temperature fractional crystallization and vacuum distillation (21). 12-Ketostearic acid, m.p. 81.0-81.4°, was prepared by the chromic acid oxidation of 12-hydroxystearic acid, by the method of Willstätter (28), except that the reaction mixture was poured into a large quantity of water to precipitate the product, which was first recrystallized from acetic acid and then from 95% ethyl alcohol. Tetralin hydroperoxide (10), m.p. 54.0-54.5°, was prepared by passing oxygen through pure tetralin (7) at 70° for 70 hours. Unreacted tetralin was removed by distillation at 0.2-0.4 mm. to a maximum pot temperature of 70°, and the residue was then recrystallized at -50° from toluene (2 ml. of solvent/g. of solute). Tertiary butyl hydroperoxide (14), b.p. 34.5-35°/13 mm. and n_D^{25} 1.3987, was prepared by vacuum distillation of the commercial product obtained from the Union Bay State Co.⁴ Di-tertiary butyl peroxide (15), b.p. 38.3°/51 mm. and n_D^{25} 1.3865, was prepared by vacuum distillation of the 98% grade obtained from the Shell Development Company. Cumene hydroperoxide, b.p. 65°/0.1 mm. and n_D^{25} 1.5221, was prepared by vacuum distillation of the commercial product obtained from the Hercules Powder Co. Cyclohexene hydroperoxide, b.p. 40-41°/0.2 mm., d_4^{30} 1.0546, d_4^{20} 1.0676, n_D^{30} 1.4822, and n_D^{20} 1.4892, was prepared from cyclohexene (17) by the procedure of Criegee, Pilz, and Flygare (3). Cyclohexene-3-ol, b.p. 60-65°/12 mm., was prepared by treating an acetic acid solution of cyclohexene hydroperoxide (11 g.) with excess 50% aqueous potassium iodide for one hour, extracting the product with chloroform, washing the chloroform solution free of acid with water, drying over calcium sulfate, and then fractionally distilling the solution under vacuum. Benzoyl peroxide was the Eastman Kodak White Label Grade. Methyl oleate hydroperoxide, purity about 70%, was obtained from C. E. Swift, National Cottonseed Products Association Fellow, Southern Regional Research Laboratory. Acetophenone, mesityl oxide, methyl isobutyl ketone, dioxane, and cyclohexanone were purified by efficient fractional distillation of the purest commercial grades.

Analytical Methods. Acid Number. Approximately 1-g. samples were dissolved in 75 ml. of neutralized 95% ethyl alcohol and titrated with 0.1 N aqueous sodium hydroxide to a phenolphthalein end-point. When only a small quantity of material was available, the weight of the sample was reduced considerably, and a semi-micro burette was employed.

$$\text{Acid Number} = \frac{\text{ml. of NaOH} \times N \times 56.10}{\text{weight of sample}}$$

Saponification Number. Approximately 0.5-g. samples were weighed into 250-ml. Kimble glass Erlenmeyer flasks and dissolved in 25 ml. of aldehyde-free 95% ethyl alcohol. Exactly 25 ml. of approximately 0.2 N alcoholic potassium hydroxide solution was added, the mixture was refluxed for one hour on the steam bath, and the excess alkali was titrated with 0.1 N aqueous hydrochloric acid to a phenolphthalein end-point. Blank determinations were carried out in the same way.

⁴ The Bureau of Agricultural and Industrial Chemistry, United States Department of Agriculture, does not recommend products or companies mentioned in preference to others not mentioned.

RATE OF DECOMPOSITION OF HYDROXYLAMINE REAGENT AT 26°C

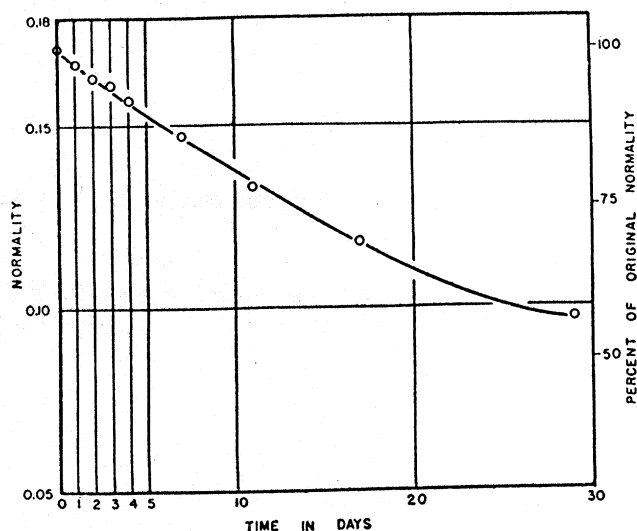


Fig. 1. Rate of decomposition of hydroxylamine reagent at 26°C.

$$\text{Saponification Number} = \frac{A \times N \times 56.10}{\text{weight of sample}}$$

A = Blank titration — ml. of HCl employed in back-titrating.

Iodine Number. The conventional one-half hour Wijs method was employed.

$$\text{Iodine Number} = \frac{A \times N \times 0.1269 \times 100}{\text{weight of sample}}$$

A = Blank titration — ml. of sodium thiosulfate employed in back-titrating.

Hydroxyl Oxygen. The procedure of Ogg, Porter, and Willits was employed (16), except that 5 ml. of the pyridine-acetic anhydride reagent was used and the sample weights were increased accordingly.

$$\text{Hydroxyl Oxygen, \%} = \frac{[B - (A - C)] \times N \times 0.016}{\text{weight of sample}} \times 100$$

B = Blank titration.

A = Ml. of NaOH employed in back-titrating.

C = Ml. of NaOH required to neutralize the acidity of the sample.

Oxirane Oxygen. The procedure of Swern, Findley, Billen, and Scanlan (20) was employed.

$$\text{Oxirane Oxygen, \%} = \frac{[B - (A - C)] \times N \times 0.016}{\text{weight of sample}} \times 100$$

B = Blank titration.

A = Ml. of NaOH employed in back-titrating.

C = Ml. of NaOH required to neutralize the acidity of the sample.

Peroxide Oxygen. Wheeler's method (26) was employed except that all precautions were taken to exclude oxygen, and a 15-minute reaction period was employed. Blank titrations, which did not exceed 0.1 ml., were also run. The percentage of peroxide oxygen includes both oxygen atoms of the peroxide group even though only one oxygen atom is active.

$$\text{Peroxide Oxygen, \%} = \frac{A \times N \times 0.016}{\text{weight of sample}} \times 100$$

A = Ml. of sodium thiosulfate employed — blank titration.

Carbonyl Oxygen. Since the determination of carbonyl oxygen on fatty materials and their mixed ox-

dation products has not been adequately described in the literature (12, 13), a detailed investigation of the method was made. The procedure finally adopted is described below:

Aldehyde-free Alcohol. Reflux 1 l. of 95% ethyl alcohol for 30 minutes with 10 g. of potassium hydroxide and 6 g. of granulated metallic aluminum. Distill and discard first and last 50 ml. of distillate.

Hydroxylamine Reagent—Approximately 0.2 N. Dissolve 20 g. of hydroxylamine hydrochloride (Eastman Kodak White Label) in 40 ml. of distilled water and then add 400 ml. of aldehyde-free alcohol. Add 300 ml. of 0.5 N alcoholic potassium hydroxide to this solution, mix well, and filter. The filtrate consists of a solution of hydroxylamine hydrochloride and free hydroxylamine. This solution is somewhat unstable at room temperature, as shown in Fig. 1, and is preferably freshly prepared each day.

Procedure. Weigh sample, in accordance with expected percentage of carbonyl oxygen as shown in the following table, into a 250-ml. iodine flask with special sidearms to accommodate electrodes (16). Add exactly 25 ml. of hydroxylamine reagent from an automatic pipette, stopper the sidearms with ground glass stoppers, and reflux the solution on the steam

Expected Per Cent of Carbonyl Oxygen	Maximum Weight of Sample
1	3.2
2	1.6
4	0.8
8	0.4
16	0.2

bath for 10 to 30 minutes (low molecular weight aldehydes and ketones, 10 minutes; long-chain compounds, 30 minutes). Allow the solution to cool to room temperature, insert the electrodes through the sidearms, and titrate with 0.1 N hydrochloric acid to a pH of 3.0, employing a pH meter. (Fig. 2 is a typical titration curve). Shortly before the end-point is reached, wash the sides of the flask and the electrodes with 10 ml. of distilled water. At least two blank determinations, which must check within 0.1 ml., should be made. (With compounds insoluble in the cooled solution, provision should be made for stirring during the titration.)

$$\text{Carbonyl Oxygen, \%} = \frac{A \times N \times 0.016}{\text{weight of sample}} \times 100$$

A = Blank titration — ml. of HCl employed in back-titrating.

TITRATION CURVE FOR CARBONYL OXYGEN DETERMINATION

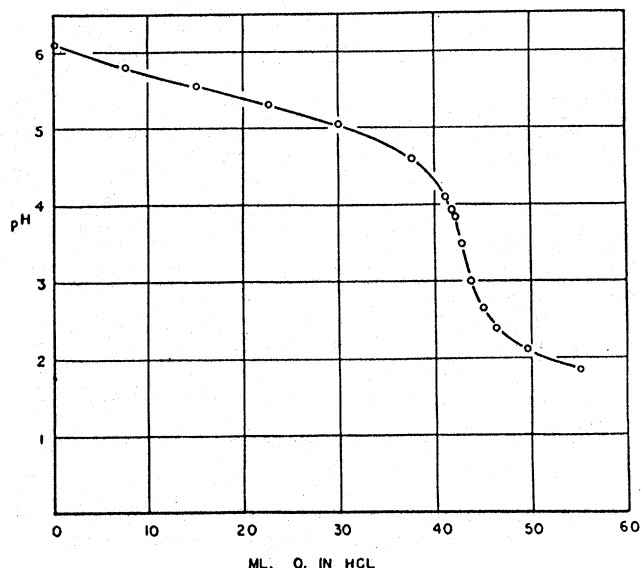


Fig. 2. Titration curve for carbonyl oxygen determination.

The results obtained when this method was applied to some typical carbonyl compounds are shown in Table I.

TABLE I
Analysis of Purified Carbonyl Compounds

	Carbonyl Oxygen, %	
	Calcd.	Found
Acetophenone.....	13.33	13.34
Mesityl oxide.....	16.32	15.97
Methyl-isobutyl ketone.....	15.98	15.35
Cyclohexanone.....	16.31	16.54
12-Ketostearic acid.....	5.36	5.33

Discussion

The results obtained when the analytical methods just discussed were applied to a series of purified compounds are shown in Table II. This table shows several important points. a) The analytical methods are reliable provided that peroxide and oxirane groups are absent. Thus, the ester, carboxyl, primary and secondary hydroxyl, carbonyl, α -glycol, ether, and the isolated and allylic types of ethylenic groups do not interfere. No conjugated polyunsaturated aliphatic compounds (eleostearic acid, etc.) were studied since it is well known that these do

TABLE II
Analysis of Purified Compounds

Compound	Acid Number		Saponif. Number		Iodine No. (Wjs, ½ hr.)		Oxirane Oxygen, %		Hydroxyl Oxygen, %		Carbonyl Oxygen, %		Peroxide Oxygen, % ¹	
	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
Methyl Oleate.....	0	0	189.2	189.8	85.6	84.4	0	0	0	0.18	0	0.15	0	0
9,10-Epoxyoctadecaric Acid.....	188.0	187.7	188.0	188.2	0	0	5.36	5.37	0	2.20	0	0	0	0
Methyl Ricinoleate.....	0	0	179.5	179.8	81.2	82.5	0	0	5.12	5.18	0	0.12	0	0
12-Ketostearic Acid.....	188.0	188.3	188.0	188.0	0	3.2	0	0.14	0	0	5.36	5.33	0	0
9,10-Dihydroxyoctadecaric Acid.....	177.2	177.7	177.2	177.7	0	0	0	0	10.74	10.78	0	0	0	0
Oleyl Alcohol.....	0	0	0	0	94.5	92.0	0	0	6.33	6.28	0	0	0	0
Tetralin Hydroperoxide.....	0	0	0	20.4	0	142.2	0	0	0	0	0	13.6	19.48	19.40
Tertiary Butyl Hydroperoxide.....	0	0	0	48.63	0	0	0	0	0	15.3	0	16.18	35.50	34.60
Di-tertiary Butyl Peroxide.....	0	0	0	0	0	0	0	0	0	0	0	0	21.88	0
Cumene Hydroperoxide.....	0	0	0	28.34	0	58.62	0	0	0	0.75	0	11.12	21.04	20.96
Cyclohexene Hydroperoxide.....	0	1.8	0	197	222.4	201.8	0	0.54	0	0.91	0 ²	28.05	27.04
Cyclohexene-3-ol.....	0	2.4	0 ²	258.6	229.9	0	0.64	16.31	14.17	0 ²	0	0.03
Benzoyl Peroxide.....	0	1.0	?	491.516	0	0	0	0	0	0	0	19.24	13.22	13.10
Dioxane.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0

¹ Includes both oxygen atoms of the peroxide group. ² Material used up.

TABLE III

$$^1 \text{Oxygen in carboxyl and ester groups, \%} = \frac{32 \times \text{Saponification Number}}{56104} \times 100.$$
² By combustion.³ Calculated from analyses by addition.

Although pure peroxide and oxirane compounds interfere with some of the analytical procedures, it does not necessarily follow that they would be harmful in the proportions encountered in oxidation reaction mixtures. On the other hand, in combination with many functional groups, the errors might actually be enhanced. For these reasons two synthetic mixtures were prepared, one containing approximately 10%, the other approximately 20%, each of a peroxide and an oxirane compound, and the analytical procedures described were carried out on these mixtures. The composition of the mixtures and the calculated and found analytical values are shown in Table III. In mixture 1, which contained only 10% of peroxide, both the iodine number and the carbonyl oxygen analyses were in serious error. Hydroxyl oxygen was slightly high, as a result of the presence of oxirane oxygen, but the error was not considered sufficiently large (0.17 unit %) to cause any concern. In mixture 2, which contained 20% of peroxide, both the iodine number and carbonyl oxygen analyses showed tremendous deviations from the calculated values, and the duplication of results was poor. Hydroxyl oxygen was approximately 0.36 unit per cent high, but in complex mixtures we have been unable to devise a procedure for reducing the error. A procedure for obtaining theoretical hydroxyl oxygen analyses in simple mixtures which contain much higher percentages of oxirane compounds than mixture 2 is described later. It is essential to reduce the peroxides in mixtures containing significant quantities in order to obtain reasonably good agreement between total oxygen determined by combustion and total oxygen calculated by addition of the individual oxygen analyses as well as to ensure reliable iodine and saponification numbers.

Procedure Employed for Reduction of Peroxides

Five g. of sample are dissolved in 100 ml. of diethyl ether in an Erlenmeyer flask, and the flask is placed in an ice-water bath. Thirty ml. of a 10% aqueous solution of sodium bisulfite are slowly added, and the mixture is then stirred occasionally at room temperature over a four-hour period. The aqueous layer is separated and discarded, and the ether solution is washed several times with water. The ether solution is dried over anhydrous calcium sulfate and filtered, and the ether is separated from the

filtrate by evaporation under vacuum in a nitrogen atmosphere.

Repetition of the carbonyl oxygen analyses on mixtures 1 and 2, after reduction of the peroxide, yielded 1.03 and 1.38% carbonyl oxygen, respectively. These values are slightly higher than the calculated values although not nearly so high as were obtained for the unreduced samples. As mentioned earlier, in the reduction of purified peroxides, some carbonyl group was invariably formed, thus accounting for the high values obtained for the mixtures. The total of the oxygen analyses, however, for mixtures 1 and 2, employing the carbonyl oxygen values obtained for the reduced samples, were 13.7 and 16.8%, respectively (calculated, 13.2 and 15.5%). Furthermore, the iodine numbers of the reduced samples were 62.5 and 38.5, respectively (calculated, 59 and 36), values in good agreement with those calculated. To determine whether the reduction step caused any undesirable changes in mixtures 1 and 2 they were completely reanalyzed for the various functional groups after reduction of the peroxide. The results are shown in Table IV. The agreement

TABLE IV
Analysis of Reduced Mixtures

Mixture 1		Mixture 2	
	Found		Found
Acid Number	39.5	Acid Number	67.1
Saponification Number	178.5	Saponification Number	150.1
Iodine Number	62.5	Iodine Number	38.5
Oxirane Oxygen, %	0.35	Oxirane Oxygen, %	1.11
Hydroxyl Oxygen, %	1.25 ¹	Hydroxyl Oxygen, %	3.06 ²
Carbonyl Oxygen, %	1.03	Carbonyl Oxygen, %	1.38
Peroxide Oxygen, %	0	Peroxide Oxygen, %	0.16
Total Oxygen, %	12.8 ³	Total Oxygen, %	15.3 ⁴

¹ Calculated, 1.49.

² Calculated, 13.2.

³ Calculated, 3.24.

⁴ Calculated, 15.5.

between the calculated and found values for total oxygen in the reduced samples is good.

In our studies of oxidation mechanism we determine acid number and peroxide, oxirane, and hydroxyl oxygen on unreduced samples. If the samples contain peroxide, the remaining analyses are carried out on reduced samples. Hydroxyl oxygen values obtained on reduced samples cannot be corrected to the unreduced basis because it is not known exactly what percentage of the peroxide group is reduced to the hydroxyl group. If peroxide oxygen is low, iodine and saponification numbers are determined on unreduced samples; otherwise the peroxides are destroyed first, and a correction is made to the unreduced basis for the change in molecular weight caused by the reduction of the peroxide group. Of interest are the results obtained in the analysis of methyl oleate hydroperoxide (estimated purity about 70%). As determined by the techniques suggested in this paper, the sum of the individual values for oxygen was 19.6% (found by combustion, 20.0%) whereas the sum of the oxygen values was approximately 30% when the peroxide was not reduced.

When the value for oxirane oxygen exceeds approximately 1.5%, significant errors occur in the determination of hydroxyl oxygen. Correct hydroxyl oxygen values are obtained by converting the oxirane group in an accurately weighed sample to the chlorohydroxy group (18) and analyzing the resulting chlorohydroxy compound for hydroxyl oxygen (16). Since the oxirane group is quantitatively converted to the chloro-

hydroxy group and the chlorohydroxy compound can be isolated quantitatively, the true hydroxyl content of the original material can be obtained by subtracting the percentage of oxirane oxygen from the percentage of hydroxyl oxygen obtained by analysis of the chlorohydroxy compound. Since all the chlorohydroxy compound obtained from a known weight of oxirane compound is analyzed, no corrections need be made for changes in molecular weight, and the weight of the sample to be used in the calculation of hydroxyl oxygen is the weight of the oxirane compound.

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Summary

Conventional analytical procedures employed in oxidation reactions for the quantitative determination of functional groups have been applied to a series of pure compounds as well as to two synthetic mixtures and to methyl oleate hydroperoxide (estimated purity, 70%). In the absence of peroxide and oxirane groups the analytical procedures are reliable. When peroxides are present, unusually high and variable values for carbonyl oxygen are obtained, and the iodine and saponification numbers are generally unreliable. Determination of hydroxyl oxygen is interfered with by large proportions of oxirane compounds but apparently not by peroxides. Determination of acid number and peroxide and oxirane oxygen is reliable in the presence of all the other functional groups investigated. Techniques for the accurate determination of functional groups when peroxide and oxirane groups are present are described.

A modified procedure for the determination of carbonyl oxygen is reported.

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